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# ANTIOXIDANT AND ACETYLCHOLINESTERASE INHIBITORY PROPERTIES OF DIVERSE γ-PYRIDINYL AMINE DERIVATIVES

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## ABSTRACT

Diverse acyclic and cyclic γ-pyridinyl amines derivatives were easily *de novo* prepared once more using respective aldimines derived from commercially available γ-pyridine carboxyaldehyde and anilines. Obtained secondary amines were tested as possible antioxidant agents with acetylcholinesterase (AChE) inhibitory activity. Both types of amines exhibited a good antioxidant activity and moderate AChE inhibitory properties. Two compounds 4-methoxy-*N*-(pyridin-4-ylmethyl) aniline **3c** and 4-methyl-2-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline **5a**, showed highest the Trolox® Equivalent Antioxidant Capacity (TEAC) values (TEAC = 1.896 and 1.252, respectively), superior than those of α-tocopherol, well-known antioxidant (TEAC = 0.888). The best AChE inhibitor with $IC_{50}$ of 48.3 μM (8.9 μg/mL) was the *N*-(pyridin-4-ylmethyl) aniline. From comparative antioxidant/AChE tests, it was identified the 4-methoxy-*N*-(pyridin-4-ylmethyl) aniline **3c** with suitable bio-parameters for design and development of new pyridine-based molecules with dual activity, antioxidant/AChE inhibitory capacities.

**KEYWORDS:** **γ-Pyridinyl amines, antioxidant agents, AChE inhibitory properties**

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## INTRODUCTION

Secondary amines that contain aromatic planar rings (e.g. aryl, pyridinyl, furyl, naphthyl, etc.) are extensively used in the chemical industry as rudimentary intermediates to prepare pharmaceuticals, agrochemicals, and fine chemicals[1-5]. In particular, pyridine amides have been found to exhibit broad spectrum insecticidal, acaricidal and nematicidal activity through inhibition of mitochondrial electron transport (MET) at site I[6]. Furthermore, they are one of the most common structural features of naturally occurring biologically active compounds[7] playing a key role in drug discovery. Although diverse pyridinyl amines were well studied[8], this type of compounds still have demonstrated to be interesting pharmacological models in biomedicine studies[2]. In particular, heterocyclic amines including pyridinyl amines have been used as simple molecular models for the search of new antioxidant agents. The biological significance of these agents gained importance recently due to their key role in the inactivation of reactive oxygen species (ROS) that are linked to different pathological processes ranking from diabetes, cardiovascular disease, and carcinogenesis to Alzheimer's dementia[9]. So, chemistry and biology of pyridine-based compounds with antioxidant properties have received substantial consideration from both theoretical and practical points of view. As a continuation of our efforts in developing new nitrogen-molecules for various pharmacological applications, *e.g.*, antifungal, anticancer, antioxidant agents[10-14], we are now presenting new data on the biological properties of selected 11 γ-pyridinylamines derivatives, belonging to three types of acyclic and cyclic compounds, that were prepared from commercially available γ-pyridinecarboxyaldehyde and diverse anilines. Two biological tests, free radical scavenging capacity using the Trolox® Equivalent Antioxidant Capacity (TEAC) value, obtained by the $ABTS^{+\bullet}$ radical-cation discoloration method and acetylcholinesterase (AChE) inhibition assays were performed to evaluate possible biological activities of above mentioned series. Both methods are commonly used in the search of new pharmacological agents for prevention and treatment of diseases associated to oxidative stress. Recent studies have found that the inflammatory processes are directly associated with Alzheimer's disease since ROS are responsible for cell damage at the structural level and act as secondary messengers in inflammation[15,16]. Bioassays testing antioxidant activity and AChE inhibitory properties of small heterocyclic molecules could help to discover suitable molecular scaffolds to construct new agents for treatment of diseases related to oxidative stress. So, in our present study we have investigated the ability of prepared γ-pyridinyl amines derivatives to capture ROS and inhibit AChE.

## MATERIALS AND METHODS

### *Chemistry*

IR spectra were recorded on a LumexInfralum FT-02 spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were measured on a Bruker AM-400 spectrometer (400 MHz or 300 MHz $^1H$ NMR and 100 MHz or 75 MHz $^{13}C$ NMR), using $CDCl_3$ as the solvent. TMS was used as an internal standard. Chemical shifts (δ) and *J* values are reported in ppm and Hz, respectively. A Hewlett Packard 5890a Series II Gas Chromatograph interfaced to an HP 5972 Mass Selective Detector with an HP MS ChemStation Data system was used for MS identification at 70 eV using a 60 m capillary column coated with HP-5 [5% phenylpoly(dimethylsiloxane)]. Melting points were measured on a Fisher Johns melting point apparatus. The reaction progress was monitored using thin layer chromatography on a Silufol UV 254 TLC aluminum sheets. Column chromatography was carried out using silica gel (230-400 mesh). All reagents were purchased from Sigma and Aldrich Chemical Co and used without further purification. Synthesis of the secondary amines **3-5** were performed according to literature reports[11-14,17]. Their spectral data were identical to those published in our works.

#### *N-(Pyridin-4-ylmethyl)aniline 3a*

$^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 4.37 (2H, s, $CH_2$-N), 6.58 (2H, dd, *J* = 8.5, 0.8 Hz, 2(6)-$H_{Ph}$), 6.74 (1H, t, *J* = 7.4 Hz, 4-$H_{Ph}$), 7.17 (2H, td, *J* = 7.4, 1.0 Hz, 3(5)-$H_{Ph}$), 7.29 (2H, d, *J* = 5.9 Hz, 3(5)-$H_{Py}$), 8.55 (2H, dd, *J* = 6.0, 1.6 Hz, 2(6)-$H_{Py}$). $^{13}C$ NMR (75 MHz), δ (ppm): 47.0, 112.8 (2C), 118.0, 122.0 (2C), 129.3 (2C), 147.4, 149.0, 149.8 (2C). GC-MS: $t_R$ 15.08 min., 184 ($M^+$, 100), 106 (80), 92 (11), 79 (20), 77 (19), 65 (14), 51 (12).

#### *4-Methyl-N-(pyridin-4-ylmethyl) aniline 3b*

$^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.23 (3H, s, $CH_3$), 4.15 (1H, bs, N-H), 4.36 (2H, s, $CH_2$-N), 6.50 (2H, d, *J* = 8.4 Hz, 2(6)-$H_{Ar}$), 6.98 (2H, d, *J* = 8.3 Hz, 3(5)-$H_{Ar}$), 7.31 (2H, d, *J* = 4.2 Hz, 3(5)-$H_{Py}$), 8.54 (2H, dd, *J* = 4.2, 1.5 Hz, 2(6)-$H_{Py}$).$^{13}C$ NMR (75 MHz), δ (ppm): 20.3, 47.4, 112.9, 113.0, 122.1, 122.2, 123.4, 128.0, 129.7, 129.8, 145.1, 149.4, 149.5. HRMS (70 eV, EI): found 198.116886. Calc. For $C_{13}H_{14}N_2$ [$M^+$]: 198.115699.

#### *4-Methoxy-N-(pyridin-4-ylmethyl) aniline 3c*

$^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 3.71 (3H, s, $OCH_3$), 4.08 (1H, bs, N-H), 4.30 (2H, s, $CH_2$-N), 6.53 (2H, d, *J* = 8.7 Hz, 2(6)-$H_{Ar}$), 6.75 (2H, d, *J* = 8.7 Hz, 3(5)-$H_{Ar}$), 7.27 (2H, d, *J* = 5.0 Hz, 3(5)-$H_{Py}$), 8.52 (2H, d, *J* = 5.3 Hz, 2(6)-$H_{Py}$). $^{13}C$ NMR (100 MHz), δ (ppm): 47.7, 55.6, 113.9, 114.8, 122.0, 141.5, 149.1, 149.8, 152.3. GC-MS: $t_R$ 19.90 min., 214 ($M^+$, 100), 199 (37), 136 (15), 122 (60), 92 (41), 65 (13).

#### *3-Methyl-N-(pyridin-4-ylmethyl) aniline 3d*

$^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.24 (6H, s, 3(5)-$CH_3$), 4.12 (1H, bs, N-H), 4.39 (2H, s, $CH_2$-N), 6.24 (2H, s, 2(6)-$H_{Ar}$), 6.43 (1H, s, 4-$H_{Ar}$), 7.33 (2H, bd, *J* = 4.4 Hz, 3(5)-$H_{Py}$), 8.57 (2H, dd, *J* = 4.4, 1.5 Hz, 2(6)-$H_{Py}$). $^{13}C$

NMR (100 MHz), δ (ppm): 47.7, 55.6, 113.9, 114.8, 122.0, 141.5, 149.1, 149.8, 152.3. GC-MS: $t_R$ 18.52 min., 212 ($M^+$, 100), 134 (82), 105 (8), 91 (14), 79 (13), 77 (15).

***N-(1-(Pyridin-4-yl)but-3-en-1-yl) aniline 4a***

$^1$H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.43-2.66 (2H, m, -$CH_2$), 4.19 (1H, bs, N-H), 4.38 (1H, dd, *J* = 7.9, 5.0 Hz, -CH), 5.18-5.24 (2H, m, =$CH_2$), 5.66-5.80 (1H, m, =CH), 6.45 (2H, d, *J* = 8.5 Hz, 2(6)-$H_{Ph}$), 6.69 (1H, tt, *J* = 7.3 Hz, 4-$H_{Ph}$), 7.10 (2H, td, *J* = 7.7, 1.0 Hz, 3(5)-$H_{Ph}$), 7.31 (2H, dd, *J* = 4.4, 1.6 Hz, 3(5)-$H_{Py}$), 8.56 (2H, dd, *J* = 5.0, 1.6 Hz, 2(6)-$H_{Py}$). $^{13}$C NMR (100 MHz), δ (ppm): 42.4, 56.2, 113.4 (2C), 117.9, 119.1, 121.6 (2C), 129.1 (2C), 146.6, 133.5, 150.0 (2C), 152.8. GC-MS: $t_R$ 17.27 min., 224 ($M^+$, 3), 183 (100), 77 (11), 51 (4).

***4-Methyl-N-(1-(pyridin-4-yl)but-3-en-1-yl) aniline 4b***

$^1$H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.20 (3H, s, $CH_3$), 2.43-2.64 (2H, m, -$CH_2$), 4.09 (1H, br. s, N-H), 4.34 (1H, dd, *J* = 7.7, 5.0 Hz, -CH), 5.18 (1H, s, $CH_A$=), 5.21 (1H, d, *J* = 7.8 Hz, $CH_B$=), 5.68-5.78 (1H, m, =CH), 6.38 (2H, d, *J* = 8.4 Hz, 2(6)-$H_{Ar}$), 6.91 (2H, d, *J* = 8.0 Hz, 3(5)-$H_{Ar}$), 7.31 (2H, dd, *J* = 4.7, 1.1 Hz, 3(5)-$H_{Py}$), 8.55 (2H, dd, *J* = 4.6, 1.4 Hz, 2(6)-$H_{Py}$). $^{13}$C NMR (100 MHz), δ (ppm): 20.3, 42.4 (-), 56.4 (+), 113.4 (2C, +), 119.0 (-), 121.6 (2C, +), 127.0, 129.6 (2C, +), 133.6 (+),144.3, 150.0 (2C, +), 152.9. GC-MS: $t_R$ 19.27 min., 238 ($M^+$, 4), 197 (100), 91 (8), 65 (4).

***4-Methoxy-N-(1-(pyridin-4-yl)but-3-en-1-yl) aniline 4c***

$^1$H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.41-2.61 (2H, m, -$CH_2$), 3.68 (3H, s, $OCH_3$), 4.29 (1H, dd, *J* = 8.0, 5.0 Hz, -CH), 5.16 (1H, s, $CH_B$=), 5.20 (1H, d, *J* = 6.0 Hz, $CH_A$=), 5.67-5.77 (1H, m, =CH), 6.40 (2H, d, *J* = 8.9 Hz, 2(6)-$H_{Ar}$), 6.68 (2H, d, *J* = 8.9 Hz, 3(5)-$H_{Ar}$), 7.29 (2H, d, *J* = 6.0 Hz, 3(5)-$H_{Py}$), 8.53 (2H, dd, *J* = 4.5, 1.5 Hz, 2(6)-$H_{Py}$). $^{13}$C NMR (100 MHz), δ (ppm): 42.4, 55.6, 57.0, 114.5 (2C), 114.7 (2C), 119.0, 121.6 (2C), 133.6, 140.8, 149.8 (2C), 152.2, 153.1. GC-MS: $t_R$ 21.32 min., 254 ($M^+$, 5), 213 (100), 198 (4), 169 (7).

***3-Methyl-N-(1-(pyridin-4-yl)but-3-en-1-yl) aniline 4d***

$^1$H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.15 (6H, s, 3(5)-$CH_3$), 2.41-2.62 (2H, m, -$CH_2$), 4.07 (1H, bs, N-H), 4.35 (1H, dd, *J* = 7.7, 5.0 Hz, -CH), 5.20-5.15 (2H, m, $CH_2$=), 5.65-5.76 (1H, m, =CH), 6.08 (2H, s, 2(6)-$H_{Ar}$), 6.34 (1H, s, 4-$H_{Ar}$), 7.29 (2H, dd, *J* = 4.7, 1.3 Hz, 3(5)-$H_{Py}$), 8.54 (2H, dd, *J* = 4.5, 1.5 Hz, 2(6)-$H_{Py}$). $^{13}$C NMR (100 MHz), δ (ppm): 21.4 (2C), 42.4, 56.2, 111.3 (2C), 119.0, 119.9, 121.5 (2C), 133.6, 138.8 (2C), 146.8, 150.0 (2C), 152.9. GC-MS: $t_R$ 19.99 min., 252 ($M^+$, 3), 211 (100), 105 (5), 77 (7).

***4-Methyl-2-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline 5a***

*cis* isomer:$^1$H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.34 (3H, d, *J* = 6.8 Hz, 4-$CH_3$), 1.70 (1H, c, *J* = 12.7 Hz, 3-Ha), 2.11 (1H, ddd, *J* = 12.9, 4.3, 3.0 Hz, 3-He), 3.13 (1H, sp, *J* = 6.0 Hz, 4-H), 4.00 (1H, s, NH), 4.47 (1H, dd, *J* = 11.3, 2.8 Hz, 2-H), 6.56 (1H, dd, *J* = 7.9, 0.9 Hz, 8-H), 6.74 (1H, td, *J* = 7.4, 0.9 Hz, 6-H), 7.03 (1H, t, *J* = 7.6 Hz, 7-H), 7.18 (1H, d, *J* = 7.7 Hz, 5-H), 7.35 (2H, dd, *J* = 4.7, 1.3 Hz, 3(5)-$H_{Py}$), 8.58 (2H, dd, *J* = 4.5, 1.5 Hz, 2(6)-$H_{Py}$). $^{13}$C NMR (100 MHz), δ (ppm): 20.1, 31.1, 41.2, 56.0, 114.4, 118.2, 121.6 (2C), 126.0, 126.9, 127.1, 144.1, 150.2 (2C), 153.4. GC-MS: $t_R$ 20.06 min., 224 ($M^+$, 91), 209 (46), 146 (100), 130 (24), 117 (15), 104 (7), 91 (11), 77 (14), 51 (8).

***4,6-Dimethyl-2-(pyridin-4-yl)-1,2,3,4-tetra hydroquinoline 5b***

*cis* isomer: $^1$H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.34 (3H, d, *J* = 6.8 Hz, 4-$CH_3$), 1.69 (1H, c, *J* = 11.6 Hz, 3-Ha), 2.10 (1H, ddd, *J* = 12.9, 5.3, 2.1 Hz, 3-He), 2.26 (3H, s, 6-$CH_3$), 3.12 (1H, sept, *J* = 6.6 Hz, 4-H), 3.86 (1H, bs, NH), 4.43 (1H, dd, *J* = 11.3, 2.6 Hz, 2-H), 6.50 (1H, d, *J* = 8.0 Hz, 8-H), 6.86 (1H, d, *J* = 8.0 Hz, 7-H), 7.00 (1H, s, 5-H), 7.35 (2H, dd, *J* = 4.5, 1.4 Hz, 3(5)-$H_{Py}$), 8.58 (2H, dd, *J* = 4.4, 1.6 Hz, 2(6)-$H_{Py}$). $^{13}$C NMR (100 MHz), δ (ppm): 20.2, 20.6, 31.1, 41.4, 56.1, 114.6, 121.6 (2C), 126.0, 127.3, 127.5, 127.6, 141.7, 150.1 (2C), 153.5. GC-MS: $t_R$ 20.78 min., 238 ($M^+$, 10), 146 (100), 131 (26), 93 (25).

***6-Methoxy-4-methyl-2-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline 5c***

*cis* isomer: $^1$H NMR ($CDCl_3$, 400 MHz). δ (ppm): 1.33 (3H, d, *J* = 6.8 Hz, 4-$CH_3$), 1.69 (1H, c, *J* = 11.6 Hz, 3-Ha), 2.11 (1H, ddd, *J* = 13.0, 5.5, 2.7 Hz, 3-He), 3.13 (1H, sp, *J* = 6.3 Hz, 4-H), 3.76 (3H, s $OCH_3$), 4.40 (1H, dd, *J* = 11.2, 2.3 Hz, 2-H), 6.56 (1H, d, *J* = 8.6 Hz, 8-H), 6.65 (1H, dd, *J* = 8.6, 2.8 Hz, 7-H), 6.79 (1H, d, *J* = 2.7 Hz, 5-H), 7.36 (2H, dd, *J* = 4.6, 1.6 Hz, 3(5)-$H_{Py}$), 8.58 (2H, dd, *J* = 4.6, 1.6 Hz, 2(6)-$H_{Py}$). $^{13}$C NMR (100 MHz) δ (ppm): 20.3, 31.3, 41.3, 55.8, 56.2, 112.6, 113.1, 115.4, 121.7 (2C), 127.4, 138.2, 150.0 (2C), 152.5, 153.5. GC-MS: $t_R$ 22.74 min., 254 ($M^+$, 100), 239 (60), 176 (61), 93 (5).

***Bioassays: Free-Radical Scavenging Activity (ABTS Assay)***

The free radical scavenging activity was performed employing modified Re' procedure[18,19]. The optimization of $ABTS^{+\bullet}$ radical-cation was carried out with 3.34 mg of potassium peroxodisulphate ($K_2S_2O_8$) and 19.60 mg of ABTS. This mixture was flasked with 5 mL HPLC water. The reaction was kept during 16 h between 10–15 °C without light. Then, an aliquot of $ABTS^{+\bullet}$ was diluted in absolute EtOH to obtain 0.700 of absorbance to 734 nm. Standard $1x10^{-3}$ M samples solutions were analyzed and then, were again diluted until that in the presence of $ABTS^{+\bullet}$ (200 μL) the inhibition corresponded to between 20–80 % of the blank absorbance. The inhibition was evaluated after 30 min and was plotted in function of the concentrations established. All of the assays were performed in triplicate. The TEAC value was determined as the relationship between the 50% inhibitory concentrations ($IC_{50}$) of Trolox® and the antioxidant in question.

***Bioassays: AChE inhibitory activity***

50 µL of a solution of the compound (at serial concentrations from $1x10^{-3}$ to $1x10^{-7}$ M), dissolved in phosphate buffered saline of pH 7.5 and 50 µL of the AChE (0.25 U/mL) were placed on a microplate. The plate was incubated at room temperature for 30 min and it was added 100 µL of substrate solution, pH 7.5 [0.04 M $Na_2HPO_4$, 2,2'-dinitro-5,5'-dithiobenzoic 0.2 mM, 0.24 mM acetylthiocholine iodide]. At five minutes into the reaction the absorbance read at 412 nm in a VERSA max microplate reader. Galantamine was used as a reference compound. Assays were performed in triplicate. The calculations of the $IC_{50}$ and graphics were performed using SoftMax Pro 5.2 software from Molecular Devices.

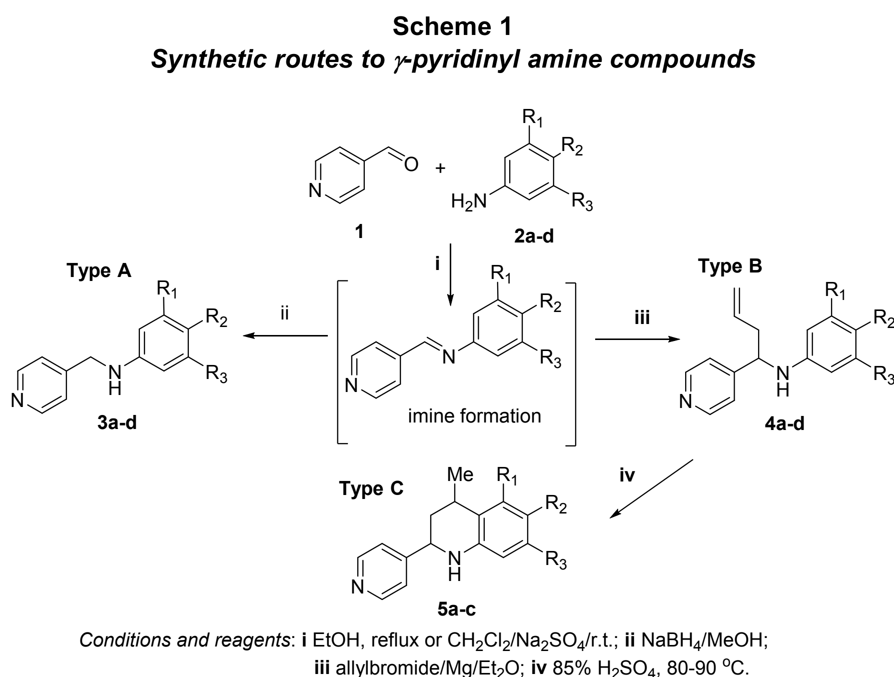
## RESULTS DISCUSSION

***Chemistry***

The tested pyridinylamine derivatives **3-5** were prepared from the commercially available 4-pyridinecarboxyaldehyde **1** and anilines **2a-d** in easy two-step or three-step synthesis protocols via initial *N*-aryl aldimine formation (Scheme 1). These aldimines, either the reduction of the C=N bond with an excess of $NaBH_4$ in methanol or by nucleophilic addition of allylmagnesium bromide (dry diethyl ether) to the C=N bond, produced the corresponding secondary *N*-(pyridin-4-ylmethyl)-*N*-aryl amines **3a-d** (type **A**) and *N*-aryl-*N*-[1-(pyridin-1-yl)but-3-enyl]amines **4a-d** (type **B**)[10-13]. The latter amines were converted in respective cyclic pyridinyl tetrahydroquinoline derivatives **5a-d** (type **C**) under gentle acid catalysis conditions (85% $H_2SO_4$, 80-90 $^oC$)[16] (Scheme 1).

**Scheme 1**

***Synthetic routes to γ-pyridinyl amine compounds***




*Conditions and reagents*: **i** EtOH, reflux or $CH_2Cl_2/Na_2SO_4$/r.t.; **ii** $NaBH_4$/MeOH; **iii** allylbromide/Mg/$Et_2O$; **iv** 85% $H_2SO_4$, 80-90 $^oC$.

These secondary amines were obtained as colored solids or viscous oils in 70-90% yields after purification using $SiO_2$ chromatography column (Table 1) and were strongly characterized by spectral data. Chemical purity of the compounds was confirmed by GC-MS as well as spectroscopic methods ($^1H$ NMR and $^{13}C$ NMR spectroscopy).The first analysis indicated that prepared compounds have a high degree of purity (> 99.5 %) while spectroscopic methods corroborated the structures of acyclic amine compounds **3,4** and structure and stereochemistry of cyclic molecules **5** that present as two diastereomers in which a *cis*-isomer (2-Py,4-Me, both equatorial) was predominant (68-91%) (Fig.1).

**Figure 1**

***Spatial representation of the cis- 2-eq,4-eq-isomers 5***

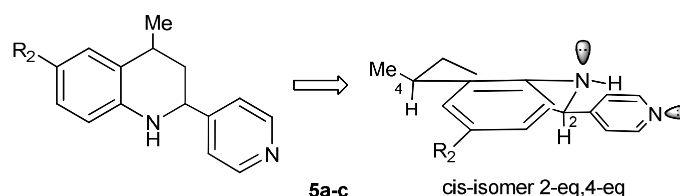


**Table 1**
**Synthetized pyridinyl amines 3-5 and their physicochemical properties**

| Comp. | $R_1$ | $R_2$ | $R_3$ | Type | Yield, % | Mp, °C | IR (KBr) $\nu_{NH}$, $cm^{-1}$ | Molecular formula | Molweight | ClogP |
|---|---|---|---|---|---|---|---|---|---|---|
| **3a** | H | H | H | **A** | 81 | 100-102 | 3263 | $C_{12}H_{12}N_2$ | 184.24 | 1.532 |
| **3b** | H | Me | H | **A** | 85 | 69-70 | 3292 | $C_{13}H_{14}N_2$ | 198.26 | 2.091 |
| **3c** | H | MeO | H | **A** | 79 | 72-73 | 3288 | $C_{13}H_{14}N_2O$ | 214.26 | 1.695 |
| **3d** | Me | H | Me | **A** | 85 | 93-94 | 3273 | $C_{14}H_{16}N_2$ | 212.29 | 2.590 |
| **4a** | H | H | H | **B** | 81 | oil | 3287 | $C_{15}H_{16}N_2$ | 224.30 | 2.475 |
| **4b** | H | Me | H | **B** | 73 | 95-96 | 3273 | $C_{16}H_{18}N_2$ | 238.33 | 2.974 |
| **4c** | H | MeO | H | **B** | 60 | oil | 3294 | $C_{16}H_{18}N_2O$ | 254.33 | 2.578 |
| **4d** | Me | H | Me | **B** | 74 | 91-92 | 3293 | $C_{17}H_{20}N_2$ | 252.35 | 3.473 |
| **5a** | H | H | H | **C** | 84 | 88-90 | 3266 | $C_{15}H_{16}N_2$ | 224.30 | 2.645 |
| **5b** | H | Me | H | **C** | 72 | 138-139 | 3273 | $C_{16}H_{18}N_2$ | 238.33 | 3.144 |
| **5c** | H | MeO | H | **C** | 54 | 113-115 | 3267 | $C_{16}H_{18}N_2O$ | 254.33 | 2.748 |

Taking into consideration that the design and development of new centrally acting antioxidant agents with AChE inhibitory properties are an important task of medicinal chemistry, we tested these molecules for scavenging capacity and anti-AChE activities.

***Biological properties***

***Free radical scavenging capacity***

To estimate the possible antioxidant capacity of pyridine amines **3–5**, the Trolox® Equivalent Antioxidant Capacity (TEAC) value, obtained by the $ABTS^{+\bullet}$ radical-cation discoloration method, was employed[18]. The Trolox® equivalent antioxidant capacity assay is a well-known method used to estimate an antioxidant substance ability to reduce the $ABTS^{+\bullet}$ radical-cation. In this assay, a tested molecule was added to a solution pre-formed out of the $ABTS^{+\bullet}$ radical-cation, and, within a fixed range of time, the $ABTS^{+\bullet}$ residual radical-cation was spectrophotometrically quantified. Consequently, using our own simple procedure in 96-well multiplate reader for radical scavenging capacity (reductive capacity), based on TEAC[19], new valuable data for tested compounds were obtained (Table 2). Analyzing results, we could observe that 4-methoxy-*N*-(pyridin-4-ylmethyl)aniline (**3c**, group **A**), and *N*-(4-methoxyphenyl)-*N*-[1-(pyridin-1-yl)but-3-enyl]amine (**4c**, group **B**) and 4-methyl-2-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline (**5a**), 4,6-dimethyl-2-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline (**5b**) or 6-methoxy-4-methyl-2-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline (**5c**) (latter three, from the group **C**) showed highest TEAC values (respectively, 1.856, 1.005, 1.252, 1.144 and 0.982), superior to the well-known antioxidant, α-tocopherol (TEAC = 0.888). These data confirm that tetrahydroquinolines (cyclic systems) with donating groups at C-6 position are more suitable scaffold for constructed novel antioxidants.

***AChE inhibitory activity***

The Ellman assay was used to test inhibition of AChE activity level[20]. This colorimetric assay is based on chromophores generated *in situ* after enzymatic cleavage of acetylthiocholine and the resulting thiocholine with Ellman's reagent, DTNB (5,5´-dithio-bis-2-nitrobenzoic acid). Evaluating results on enzymatic inhibition of pyridine molecules 3-5 and reference drug, alkaloid galantamine, we found that almost all tested molecules were able to inhibit AChE, showing micromolar $IC_{50}$ values ranging from 48.3 – 197.0 μM (8.9 - 50.1 μg/mL). Among this series, the best AChE inhibitor with $IC_{50}$ of 48.3 μM (8.9 μg/mL) was the *N*-(pyridin-4-ylmethyl) aniline **(3a**, group **A**), followed by the 4-methoxy-*N*-(pyridin-4-ylmethyl) aniline (**3c**, group **A**) ($IC_{50}$ = 61.4 μM) and *N*-phenyl-*N*-[1-(pyridin-1-yl)but-3-enyl] amine (**4a**, group **B**) ($IC_{50}$ = 94.3 μM) (Table 2). However, the molecule 3a with highest $IC_{50}$ values resulted in a fair antioxidant agent (TEAC = 0.599).

**Table 2**
***Antioxidant capacity (TEAC) and AChE inhibitory activity ($IC_{50}$) of the synthesized compounds.***

| Comp. | Antioxidant capacity | | AChE inhibitory activity | | |
|---|---|---|---|---|---|
| | TEAC (Average ± s) | RSD (%)* | $IC_{50}$(μg/mL) | $IC_{50}$ (μM) | RSD%* |
| **3a** | 0.599 ± 0.003 | 1.2 | 8.9 ± 0.2 | 48.3 ± 0.7 | 1.9 |
| **3b** | 1.016 ± 0.004 | 1.3 | 19.0 ± 0.6 | 95.7 ± 3.0 | 3.3 |
| **3c** | 1.896 ± 0.002 | 2.0 | 13.2 ± 0.3 | 61.4 ± 1.0 | 2.1 |
| **3d** | 1.055 ± 0.02 | 0.1 | 38.5 ± 1.8 | 181.0 ± 9.0 | 4.7 |
| **4a** | 0.029 ± 0.02 | 4.6 | 21.1 ± 0.03 | 94.3 ± 1.0 | 1.4 |
| **4b** | 0.017 ± 0.0001 | 2.0 | 35.4 ± 1.3 | 148.0 ± 5.0 | 3.6 |
| **4c** | 1.005 ± 0.03 | 1.2 | 50.1 ± 2.0 | 197.0 ± 8.0 | 4.0 |
| **4d** | 0.045 ± 0.0 | 2.4 | 28.9 ± 0.4 | 115 ± 2.0 | 1.6 |
| **5a** | 1.252 ± 0.01 | 0.7 | 19.4 ± 0.1 | 86.6 ± 0.5 | 0.6 |
| **5b** | 1.144 ± 0.02 | 0.1 | 16.0 ± 0.4 | 67.0 ± 1.0 | 2.2 |
| **5c** | 0.989 ± 0.04 | 2.6 | 22.2 ± 0.0 | 87.2 ± 0.2 | 0.2 |
| **α-Tocopherol** | 0.888 ± 0.01 | 1.9 | 42.0 ± 2.3 | 97.6 ± 0.7 | 1.5 |
| **Galantamine** | - | - | 0.30 ± 0.01 | 1.06 ± 0.04 | 3.5 |

***RSD %: Relative standard deviation, n = 6.***

Comparing both biological screenings, we found that the best molecule from these series was the simple pyridin-4-ylmethyl-aniline (**3c**, group **A**) that possesses the highest TEAC values and moderated $IC_{50}$ values (Table 2).

# CONCLUSION

In summary, we have analyzed the free radical scavenging capacity and AChE inhibitory properties of some pyridinyl amine derivatives prepared from available aldimines. During analysis, it was identified the 4-methoxy-*N*-(pyridin-4-ylmethyl) aniline **3c** with suitable bio-parameters for design and development of new pyridine-based dual activity, antioxidant/AChE inhibitory capacities.

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# REFERENCES


1. Seayad A., Ahmed M., Klein H., Jackstell R., Gross T., Beller M. Internal Olefins to Linear Amines. Science, 297 (5587): 1676-1678, (2002).
2. Kleemann A., Engel J., Kutscher B., Reichert D. Pharmaceutical Substances: Syntheses, Patents, Applications, 4ed., Thieme Verlag: Stuttgart, 1-2521, (2001).
3. Evers A., Hessler G., Matter H., Klabunde T. Virtual Screening of Biogenic Amine-Binding G-Protein Coupled Receptors: Comparative Evaluation of Protein- and Ligand-Based Virtual Screening Protocols. J Med Chem, 48 (17): 5448-5465, (2005).
4. Salvatore R.N., Yoon C.H., Jung K.W. Synthesis of Secondary Amines. Tetrahedron, 57 (37): 7785-7811, (2001).
5. Henkel T., Brunne R.M., Mueller H., Reichel F. Statistical Investigation into the Structural Complementarity of Natural Products and Synthetic Compounds. Angew Chem Int Ed, 38 (5): 643-647, (1999).
6. Johnson P.L., Hackler R.E., Sheets J.J., Worden T., Gifford J. Synthesis and Insecticidal Activity of *N*-(4-Pyridinyl and Pyrimidinyl) phenyl acetamides. In: D.R. Baker, J.G. Fenyes, G.S. Basarab, D.A. Hunt (eds.), Synthesis and Chemistry of Agrochemicals V., pp. 136-146, (1998)
7. Abdel-Magid A.F., Mehrman SJ. A Review on the Use of Sodium Triacetoxyborohydride in the Reductive Amination of Ketones and Aldehydes. Org Process Res Dev, 10 (5): 971-1031, (2006).
8. Katrizky A.R. (ed.), Comprehensive Heterocyclic Chemistry. Vol 2, Pergamon Press, Oxford: 395, (1984).
9. Cadenas E., Packer L. (eds.), Handbook of Antioxidants: Marcel Dekker, Inc.: New York: 1-732, (2001).
10. Urbina J.M.G., Cortés J.C., Palma A., López S.N., Zacchino S.A., Enriz R.D., Ribas J.C., Kouznetsov V. Inhibitors of the Fungal Cell Wall. Synthesis of 4-Aryl-4-N-arylamine-1-butenes and Related Compounds with Inhibitory Activities on β(1,3) Glucan and Chitin Synthases. Bioorg Med Chem, 8 (4): 691-698, (2000).
11. Vargas M.L.Y., Castelli M.V., Kouznetsov V.V., Urbina G.J.M., López S.N., Sortino M., Enriz R.D., Ribas J.C., Zacchino SA. In Vitro Antifungal Activity of New Series of Homoallylamines and Related Compounds with Inhibitory Properties of the Synthesis of Fungal Cell Wall Polymers, Bioorg Med Chem, 11 (7): 1531-1550, (2003).
12. Kouznetsov V.V., Rivero-Castro J., Ochoa-Puentes C., Stashenko E.E., René-Martínez J., Ochoa C., Montoro-Pereira D., Nogal-Ruiz J.J., Fernández-Portillo C., Muelas-Serrano S., Gómez-

Barrio A., Bahsas A., Amaro-Luis J. Synthesis and Antiparasitic Properties of New 4-*N*-Benzylamino-4-Hetarylbut-1-enes. Archiv der Pharmazie, 338 (1): 32-37, (2005).

13. Kouznetsov V.V., Astudillo-Saavedra L., Vargas-Méndez L.Y., Cazar-Ramírez ME. Synthesis of Some Secondary Amine Derivatives Bearing a Heteroaryl Fragment. J Chil Chem Soc, 49 (3): 197-203, (2004).
14. Suvire D.F., Sortino M, Kouznetsov V.V., Vargas M.L.Y, Zacchino S.A, Mora-Cruz U., Enriz RD. Structure–Activity Relationship Study of Homoallylamines and Related Derivatives Acting as Antifungal Agents. Bioorg Med Chem, 14 (6): 1851-1862, (2006).
15. Stuchbury G., Münch G. Alzheimer's Associated Inflammation, Potential Drug Targets and future therapies. J Neur Transm, 112 (4): 429-453, (2005).
16. Vina J., Lloret A., Orti R., Alonso D. Molecular bases of the treatment of Alzheimer's Disease with Antioxidants: Prevention of Oxidative Stress. Mol Asp Med, 25 (1-2): 117-123, (2004).
17. Vargas-Méndez L.Y., Zacchino S., Kouznetsov VV. Synthesis of New 4-Methyl-2-(4-pyridyl)-1,2,3,4-tetrahydroquinolines as Potent Antifungal Compounds. J Braz Chem Soc, 21 (1): 105-111, (2010).
18. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. Free Rad Bio Med, 26 (9-10): 1231-1237, (1999).
19. Muñoz-Acevedo A., Vargas Méndez L., Stashenko E., Kouznetsov V. Improved Trolox® Equivalent Antioxidant Capacity Assay for Efficient and Fast Search of New Antioxidant Agents. Anal Chem Lett, 1 (1): 86-102, (2011).
20. Ellman G.L., Courtney D., Andres V. Jr, Featherstone RMA. New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochem Pharmacol, 7 (2): 88-95, (1961).